New Evidence for the Prevention of Diabetes and its Complications by LDR: Potential Clinical Application

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Background

• Distinct effects of LDR has been recognized more than 30 years, and extensively confirmed by studies in cultured cells *in vitro* and tissues *in vivo*, as shown by hormesis and adaptive response.

• However, few studies on the potential application of these LDR-induced hormesis and adaptive response in clinical setting have been explored.
Research of the adaptive response induced by low-dose radiation: where have we been and where should we go?

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Can we use the hormesis and/or adaptive response induced by LDR to clinic setting?

How does the AR dose relate to human environmental (ecological) exposure?

Humans live with a background radiation which may play an essential role in human health.
Low-dose radiation and its clinical implications: diabetes

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Induction of hormesis and adaptive response by low-dose radiation (LDR) has been extensively indicated. Adaptive response induced by LDR was not only resistant to damage caused by a subsequently high-dose radiation, but also cross-resistant to other non-radiation challenges, such as chemicals. Mechanisms by which LDR induces the preventive effect on radiation- or chemical-induced tissue damage include induced or up-regulated expression of protective proteins, such as heat shock proteins and antioxidants. Since oxidative damage to tissues is a major pathogenesis of many human diseases including diabetes, this review will summarize the available data with an emphasis of the preventive effect of LDR on the development of diabetes and the therapeutic effect of LDR on diabetic cardiovascular complications. The low-intensity or power laser (LIL or LPL) radiation on skin wound healing, which has stimulated clinical use of LIL to cure skin ulcer in diabetic patients. Mechanisms by which LDR prevents diabetes, though are unclear now, may include the induction of pancreatic antioxidants to prevent β cell from oxidative damage and immunomodulation to preserve pancreatic function. For LIL therapeutic effect on diabetic wound healing, mechanisms may include its antioxidant action, immunomodulation, cell proliferation stimulation as well as improvement of systemic and wound-regional microcirculation. Therefore, although only a few studies indicating LDR prevention of the development of diabetes, many studies have demonstrated LDR, specifically LIL, therapeutic effectiveness of diabetic wound healing. These preliminary results are
Diabetes is a metabolic disorder that is characterized by high blood glucose and either insufficient or ineffective insulin. 5.9% of the population in the United States has diabetes,
**Type 1 diabetes**
- Failure to produce insulin
- Approximately 1.7 million people with type 1 diabetes in USA.

**Type 2 diabetes**
- Insulin resistance
- Approximately 16 million people with type 2 diabetes in USA.

* However, type-1 diabetes markedly increase*
Diabetes Complications

Cardiomyopathy
Retinopathy
Nephropathy
Neuropathy
Skin/food ulcer
Protection against alloxan diabetes by low-dose 60Co gamma irradiation before alloxan administration.

Takehara Y, Yamaoka K, Hiraki Y, Yoshioka T, Utsumi K.

Center for Adult Diseases, Institute of Medical Science, Okayama, Japan.

They evaluated the protective effects of a single low-dose whole body 60Co gamma irradiation against alloxan-induced hyperglycemia in rats.

i) In rats that did not receive alloxan, the superoxide dismutase (SOD) activity in the pancreas significantly increased after irradiation at a dose of 0.5 or 1.0 Gy.

ii) In rats that received alloxan, plasma lipid peroxide levels, pancreatic lipid peroxide levels and blood glucose were increased. However, the increase in pancreatic lipid peroxide level was prevented by irradiation at a dose of 0.5 or 1.0 Gy; and the increase in blood glucose was prevented by irradiation at 0.5 Gy.

iii) After alloxan administration, degranulation was observed in beta cells, but this was prevented by low-dose irradiation at 0.5 Gy.
Nonobese diabetic (NOD) mice were treated with gamma irradiation and the progression of the disease was monitored.

Hyperglycemia was first detected at 15 weeks of age in the control NOD mice, whereas it was delayed as long as 7 weeks when the mice received a single dose of 0.5 Gy total-body irradiation between 12 and 14 weeks of age.

Both suppression of cell death by apoptosis and an increase in superoxide dismutase (SOD) activity were observed in the pancreas 1 week after irradiation.
LDR prevention of the development of diabetes in NOD mice.

Panel A represents the time point at which the first mouse from the groups with single LDR (0.5 Gy) at 12, 13 or 14 wks of age and without LDR (control group) spontaneously developed diabetes (hyperglycemia).

Results indicate that the first mouse developed diabetes is at 22 wks of age in the group of mice with LDR at 13 wks of age, which is 7 weeks later than that (15 wks of age) in control group.

Panel B represents the incidence of diabetic mice in different groups.

Results indicate that 10 % of mice with LDR at 13 wks of age developed diabetes at 24 wks of age, which is much lower than those in control and other LDR-treated groups.

Takahashi et al. (2000).
Amelioration of Type II Diabetes in \textit{db/db} Mice by Continuous Low-Dose-Rate \(\gamma\) Irradiation

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Although the mechanism underlying radiation hormesis is unclear, one of the candidate molecular targets is the radiation-induced biological response to oxidative stress. ERK1/2 kinase regulating growth factor-induced intracellular signaling is activated by low-dose radiation, which
FIG. 1. Continuous low-dose-rate γ irradiation preserves ability to secrete insulin in db/db mice. db/db mice (circles) and their normal littermates, db/m mice (squares), were continuously kept inside (940 μGy/h, closed symbols) or outside (0.064 μGy/h, open symbols) the irradiation room for 24 days, and blood glucose (panel A), blood insulin (panel B) and body weight (panel C) were determined. A glucose tolerance test (panel D) and insulin tolerance test (panel E) were carried out on day 14 and 21. Each point and bar represent the mean and SD (five mice per group). *Statistically significant compared to mice outside the irradiation room (P < 0.05).
FIG. 6. Continuous low-dose-rate γ irradiation increases SOD transcription in the pancreas of db/db mice. db/db mice (seven mice for control and six for irradiated) were continuously kept inside (174 μGy/h) or outside (0.064 μGy/h) the irradiation room. Mice were killed on day 21, and mRNA expression in the pancreas (left) and liver (right) was measured by real-time PCR. *Statistically significant (P < 0.05).
Summary & Question

- LDR prevented ALX-induced diabetes in rat model and also spontaneously developed non-obese diabetic (NOD) mouse model.
- LDR also reduce glucose level in type 2 diabetes, and preserve the insulin secretion.

Does LDR also prevent diabetic complications?
Low Dose Radiation Overcomes Diabetes-induced Suppression of Hippocampal Neuronal Cell Proliferation in Rats

We investigated the effect of low dose radiation on diabetes induced suppression of neurogenesis in the hippocampal dentate gyrus of rat. After 0.01 Gy, 0.1 Gy, 1 Gy and 10 Gy radiation was delivered, the dentate gyrus of hippocampus of streptozotocin (STZ)-induced diabetic rats were evaluated using immunohistochemistry for 5-bromo-2-deoxyuridine (BrdU), caspase-3, and terminal deoxynucleotidyl transferase-mediated nick end-labeling (TUNEL) staining. The number of BrdU positive cells in the non-diabetic rats, diabetic rats without radiation, diabetic rats with 0.01 Gy radiation, diabetic rats with 0.1 Gy radiation, diabetic rats with 1 Gy radiation and diabetic rats with 10 Gy radiation were 55.4 ± 8.5/mm², 33.3 ± 6.4/mm², 67.7 ± 10.5/mm², 66.6 ± 10.0/mm², 23.5 ± 6.3/mm² and 14.3 ± 7.2/mm², respectively. The number of caspase-3 positive cells was 132.6 ± 37.4/mm², 378.6 ± 99.1/mm², 15.0 ± 2.8/mm², 57.1 ± 16.9/mm², 191.8 ± 44.8/mm² and 450.4 ± 58.3/mm², respectively. The number of TUNEL-positive cells was 24.5 ± 2.0/mm², 21.7 ± 4.0/mm², 20.4 ± 2.0/mm², 18.96 ± 2.1/mm², 58.3 ± 7.9/mm², and 106.0 ± 9.8/mm², respectively. These results suggest low doses of radiation paradoxically improved diabetes induced neuronal cell suppression in the hippocampal dentate gyrus of rat.

Key Words: Radiation; Hippocampus; Diabetes Mellitus

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- Diabetes decreases BrdU intake in brain tissue (B)

- Single dose of 10 mGy (C) or 100 mGy (D) radiation prevents diabetic inhibition of BrdU intake.

- Diabetes increase brain tissue apoptotic cell death

- 10 mGy (C), 100 mGy (D) and even 1 Gy (E) reduced diabetic apoptotic cell death in brain tissue.
Possibilities for LDR’s prevention of diabetes and diabetic complications

LDR

- Immuno-modulation
- Antioxidant capacity
  - Stem cell stimulation & target cell proliferation
  - Systemic & wound regional microcirculation

Autoimmune reaction

STZ or ALX

ROS/RNS → Diabetes → ROS/RNS → Diabetic complications
Attenuation of diabetes-induced renal dysfunction by multiple exposures to low-dose radiation is associated with the suppression of systemic and renal inflammation

Chi Zhang,1,3 Yi Tan,2,3 Weiying Guo,4 Cai Li,3,5 Shunzi Ji,1 Xiaokun Li,1,3,6 and Lu Cai2,3,7

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Zhang C, Tan Y, Guo W, Li C, Ji S, Li X, Cai L. Attenuation of diabetes-induced renal dysfunction by multiple exposures to low-dose radiation is associated with the suppression of systemic and renal inflammation. Am J Physiol Endocrinol Metab 297: E1366–E1377, 2009. First published September 29, 2009; doi:10.1152/ajpendo.00478.2009.—Renal protection against diabetes-induced pathogenic injuries by multiple exposures to low-dose radiation (LDR) was investigated to develop a novel approach to the prevention of renal disease for diabetic subjects. C57BL/6J mice were given multiple low-dose streptozotocin (STZ; 60 × 6 mg/kg) to produce a type 1 diabetes. Two weeks after diabetes onset, some of effectively preventive or therapeutic approach for DN (11). It has recently been appreciated that systemic and renal inflammation caused by hyperglycemia and hyperlipidemia play an important role in the renal oxidative damage that initiates the development of renal pathogenesis (14, 24, 26, 30).

Inflammatory mediators, including adipokines, chemokines, adhesion molecules, and cytokines, were all found to play critical roles in the setting of DN (30). A growing body of evidence indicates that recruitment of inflammatory cells from
LDR’s prevention of renal inflammation and damage

25 mGy X-rays every two days for 2, 4, 8, 12, and 16 weeks

Harvest & examine cardiac inflammation and oxidative damage
Expression of TNF-α in kidney

0.0 0.5 1.0 1.5 2.0 2.5

2 w
4 w
8 w
12 w
16 w

abc
bc

bc

bc

bc

bc

bc

bc

bc

bc

bc
Whole Body Exposure to Low-dose Gamma Radiation Promotes Kidney Antioxidant Status in Balb/c Mice.

Pathak CM, Avti PK, Kumar S, Khanduja KL, Sharma SC.

Postgraduate Institute of Medical Education and Research.
We examined the effect of whole body low-dose gamma-irradiation on the status of the antioxidant defense system in the rodent kidneys at different time intervals. Young male Balb/c mice were exposed to whole body radiation from a (60)Co source at doses of 10, 25 and 50 cGy (48.78 cGy/min). Antioxidant status and lipid peroxidation were estimated in the kidneys at 4, 12 and 24 h after irradiation. Lipid peroxidation increased between 33% and 49% and reduced glutathione between 12% and 47% at 12 h at different radiation doses. Reduced glutathione level remained significantly (p < 0.05) elevated even at 24 h after irradiation to 25 cGy. Superoxide dismutase activity also increased by 37% at 12 h on exposure of animals to all the doses up to 50 cGy. Catalase activity increased significantly at 12 h on exposure to 10 cGy and 50 cGy. Interestingly, glutathione peroxidase activity increased by 31% at 4 h and subsequently returned to control levels at 24 h after exposure to 50 cGy. Glutathione reductase activity increased by 10-12% at 12 h after exposure to 25 cGy and 50 cGy. The results suggest that the whole body exposure of animals to gamma radiation stimulates the antioxidant defense system in the kidneys within 4 to 24 h after irradiation, at doses of 25 cGy and 50 cGy.
LDR’s prevention of cardiac inflammation and damage

Mice

25 m Gy X-rays every two days for 2, 4, 8, 12, and 16 weeks

Harvest & examine cardiac inflammation and oxidative damage
LDR’s prevention of diabetes-induced testicular apoptosis

Repetitive exposures to low-dose X-rays attenuate testicular apoptotic cell death in streptozotocin-induced diabetes rats

Hongguang Zhao, Songbai Xu, Zhicheng Wang, Yanbo Li, Wei Guo, Chenghe Lin, Shouliang Gong, Cai Li, Guanjun Wang, Lu Cai

Abstract

Testicular cell death was associated with increased mitochondrial dysfunction, shown by the decreased mitochondrial potential and increased expressions of Bax mRNA and protein. All these changes were significantly attenuated in certain extents by repetitive exposures to LDR. To investigate the mechanisms by which LDR attenuates diabetes-induced testicular apoptotic cell death, serum sex hormone (testosterone, luteinizing hormone and follicle stimulating hormone) levels, and both serum and testicu-
Does LDR also have certain therapeutic effect on diabetes-complications?
LDR’s therapeutic effect on diabetic wound healing

Non-diabetic mice with skin wound
Diabetic mice with skin wound
Diabetic mice with skin wound/LDR

75 mGy X-rays for 5 days, 2 day break & 5 days ...

60 days

Dose-Response 2010
Low-dose radiation (LDR) induces hematopoietic hormesis: LDR-induced mobilization of hematopoietic progenitor cells into peripheral blood circulation.

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OBJECTIVE: The aim of this study was to investigate the stimulating effect of low-dose radiation (LDR) on bone marrow hematopoietic progenitor cell (HPC) proliferation and peripheral blood mobilization. METHODS: Mice were exposed to 25- to 100-mGy x-rays. Bone marrow and peripheral blood HPCs (BFU-E, CFU-GM, and c-kit+ cells) were measured, and GM-CSF, G-CSF, and IL-3 protein and mRNA expression were detected using ELISA, slot blot hybridization, and Northern blot methods. To functionally evaluate LDR-stimulated and -mobilized HPCs, repopulation of peripheral blood cells in lethally irradiated recipients after transplantation of LDR-treated donor HPCs was examined by WBC counts, animal survival, and colony-forming units in the recipient spleens (CFUs-S). RESULTS: 75-mGy x-rays induced a maximal stimulation for bone marrow HPC proliferation (CFU-GM and BFU-E formation) 48 hours postirradiation, along with a significant increase in HPC mobilization into peripheral blood 48 to 72 hours postradiation, as shown by increases in CFU-GM formation and proportion of c-kit+ cells in the peripheral mononuclear cells. 75-mGy x-rays also maximally induced increases in G-CSF and GM-CSF mRNA expression in splenocytes and levels of serum GM-CSF. To define the critical role of these hematopoietic-stimulating factors in HPC peripheral mobilization, direct administration of G-CSF at a dose of 300 microg/kg/day or 150 microg/kg/day was applied and found to significantly stimulate GM-CFU formation and increase c-kit+ cells in the peripheral mononuclear cells. More importantly, 75-mGy x-rays plus 150 microg/kg/day G-CSF (LDR/15G-CSF) produced a similar effect to that of 300 microg/kg/day G-CSF alone. Furthermore, the capability of LDR-mobilize donor HPCs to repopulate blood cells was confirmed in lethally irradiated recipient mice by counting peripheral WBC and CFUs-S. CONCLUSION: These results suggest that LDR induces hematopoietic hormesis, as demonstrated by HPC proliferation and peripheral mobilization, providing a potential approach to clinical application for HPC peripheral mobilization.

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Bone marrow and blood stem cell levels

Mice

Control
G-CSF
LDR

25, 50, 75 or 100 mGy X-rays

Dose-Response 2010
LDR

- Receptors
- BM progenitor cell proliferation
- Mobilization
- Action
- G-CSF & other factors
- PB hematopoietic progenitor cells

Bone marrow

Peripheral Blood

Time

9 hr
48 hr
72 hr
**Figure 1.** Scheme of experimental procedures for evaluation of LDR-mobilized HPCs. LDR: 75 mGy X-rays; 150-G-CSF or 300-G-CSF: 150 or 300 μg/kg/day G-CSF administration; LDR/150-G-CSF: combining treatments of LDR and 150 μg/kg/day G-CSF administration. WBC: white blood cells. “3 d” in B group indicates that mice were sacrificed 3 days after LDR; “4 d” in C and D groups indicates that mice were consecutively administrated with G-CSF for 4 days and then sacrificed; “3 d & 4 d” in E group indicates that mice were consecutively administrated with G-CSF for 4 days and irradiated with LDR at 3 days prior to the last administration of G-CSF, and sacrificed after the last G-CSF administration. For BALB/C female mice, “4 d, 7 d, 10 d, 13 d, and 14 d” indicate the time (days) after HPC transplantation.
Summary

• LDR prevents type 1 diabetes, and reduce glucose level in type 2 diabetes.

• LDR can prevent diabetes-induced neuronal damage

• LDR can prevent diabetes-induced cardiac and renal inflammation and damage

• LDR can prevent diabetes-induced testicular damage via up-regulation of antioxidant levels.

• More importantly, LDR provides a therapeutic effect on diabetic wound healing.
Possible mechanisms

- LDR stimulate the target tissue antioxidant capacity (testicular antioxidants)
- LDR stimulate target tissue cell proliferation to replace the damage cells
- LDR has hypoglycemic effect in type 1 and type 2 diabetic models
- LDR stimulate stem cells to injured tissue to recover the damage tissue.
- \textit{Hormesis, adaptive response} and \textit{by-stander effects}
Possibilities for LDR’s prevention of diabetes and diabetic complications

LDR

- Immune-modulation
- Antioxidant capacity
- HSC stimulation & target cell proliferation

Autoimmune reaction
STZ or ALX

ROS/RNS

Diabetes

Systemic & wound regional microcirculation

Diabetic complications

Dose Response 2010
Is it potentially applied in clinics?

How to balance?

- LDR benefits
- LDR risks
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